

# New Studies on the Interaction Between 8-Methoxypsoralen and DNA in Vitro

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Some aspects of the interactions between DNA and 8-methoxypsoralen (8-MOP) in its ground state (complex formation) or in its excited state (photobinding) have been investigated. 8-MOP shows a low affinity towards DNA in the complex formation; this fact minimizes the possible biological consequences deriving from this interaction, when it occurs *in vivo*.

In covalent photobinding to DNA, 8-MOP forms mainly monofunctional adducts, and to a lesser extent bifunctional adducts, showing a behavior similar to that of other linearly condensed furocoumarins (psoralens); the ratio between mono- and bifunctional adducts was found to be 9:1.

The covalent photobinding to DNA does not occur at random along the macromolecule, but preferentially at the level of specific receptor sites. The regions having an alternate sequence of A-T seem to be the best receptor sites for the formation of monoadducts while the regions containing an alternate sequence of A-T and C-G appeared to be the preferential sites for the cross-linkage formation.

It is generally accepted that the molecular basis of the photobiological effects produced by furocoumarins (psoralens) consists of a cyclo-addition reaction to the pyrimidine bases of DNA, which takes place under irradiation with UVA light (320-400 nm) [1-3]. Other than *in vitro*, the photoconjugation to DNA occurs *in vivo*, in bacterial [4] as well as in Ehrlich ascites tumor cells [5], in guinea pig skin [6,7], and in other biological systems [8]. It has a marked inhibiting effect on DNA synthesis [9] and cell division [4,10]; therefore psoralens, associated with UVA irradiation, can be useful for disease conditions, such as psoriasis [11,12] and mycosis fungoides [13].

It was recently suggested that the ability of some furocoumarins to generate singlet oxygen under irradiation, by energy transfer from their triplet excited state, may have an important role in producing their photosensitized effects, through an oxidation of the cellular protein components [14,15]. However, it was demonstrated that this, although possible, takes place to such a reduced degree as to be practically negligible in comparison with the concomitant damage produced to DNA by cyclo-addition to the pyrimidine bases [16].

8-methoxypsoralen (8-MOP, xanthotoxin, Fig 1), isolated in Egypt from the seeds of *Ammi majus* in 1948 [17] and since that time used for the therapeutic treatment of vitiligo, in combination with exposure to sunlight or UV irradiation [18,19], was more recently investigated for the photochemotherapy of psoriasis (PUVA therapy) [11,12].

In spite of this long and widely diffused use, some aspects of its interactions with DNA have still not been defined exactly. Therefore, we have conducted studies so as to obtain exact data concerning the fundamental aspects of its interaction with DNA *in vitro*, both in the dark and after irradiation at 365 nm. In this paper we report on the data obtained.

## MATERIALS AND METHODS

### Furocoumarins

Psoralen, extracted from fig leaves [20], and 8-methoxypsoralen [from Chinoïn, Milan (Italy)], tritiated and purified according to a procedure described elsewhere [21], showed specific activities of 1.98 Ci/mol and 2.03 Ci/mol, respectively.

### Deoxyribonucleic Acids and Polydeoxyribonucleotides

The following samples of DNA were obtained from Sigma Chemical Co., St. Louis, U.S.A.: DNA from *Clostridium perfringens* (Cat. D 1760); DNA from *Micrococcus lysodeikticus* (Cat. D 8259); DNA from *Escherichia coli*, strain B (Cat. D 2001); DNA from calf thymus (Cat. D 1501). DNA from salmon sperm was purchased from Serva Feinbiochemia, Heidelberg, G.F.R. (Cat. 18570). Hypochromicity of the various DNA samples, determined according to Marmur and Doty [22] was higher than 40%.

Double stranded polydeoxyribonucleotides were purchased from Boehringer, Mannheim, G.F.R.: poly d(A-T).poly d(A-T) (Cat. 108740); poly d(A).poly d(T) (Cat. 108766); poly d(C-G).poly d(C-G) (Cat. 108782); poly d(C).poly d(G) (Cat. 108804) and poly d(A-C).poly d(G-T) (Cat. 174262).

### Binding Experiments

The binding process of 8-methoxypsoralen and of psoralen to calf thymus DNA was followed by means of equilibrium dialysis experiments as described elsewhere [23]; the values of  $r$ , that is the extent of furocoumarin molecules bound per nucleotide, at a free ligand concentration  $c$  (mol/l) have been evaluated according to Peacocke and Skerrett [24].

### Computation of Interaction Parameters

The method of computation involved an iterative procedure designed to satisfy the following equation of Mc Ghee and Von Hippel [25]:

$$\frac{r}{c} = K(1 - nr) \left[ \frac{1 - nr}{1 - (n-1)r} \right]^{n-1}$$

given the experimentally determined values of  $r$  and  $c$  and the initial guess of  $K$  (the intrinsic binding constant to an isolated site) and of  $n$  (the number of nucleotides occluded by a bound furocoumarin molecule). The program based on the least square method of Taylor series expansion of the above reported equation, was made to recycle until  $K$  and  $n$  changed by less than 1% and then to give the final values of  $K$  and  $n$  with a calculated binding isotherm at 5% saturation increments.

### Radiochemical Measurements

Small volumes (0.2 ml) of the solutions to be examined, diluted with 1 ml of distilled water, added to 10 ml of dioxane base scintillator (5 g P.P.O., 0.075 g P.O.P.O.P., 120 g naphthalene, dioxane up to 1000 ml of solution) were counted in a liquid scintillation spectrometer (Packard, Model 3375); the efficiency of the apparatus for counting tritium was within the range 26-34%.

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Abbreviation:

8-MOP: 8-methoxypsoralen

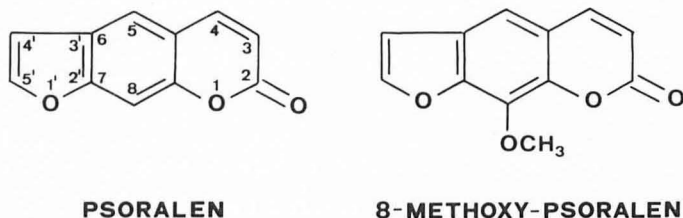


FIG 1. Molecular structures of psoralen and 8-methoxy-psoralen (8-MOP).

#### Fluorimetric Measurements

These were performed by means of a spectrophotofluorimeter (Perkin Elmer, Model PPF-044).

#### Irradiation Procedure

To aqueous solutions (0.75 mM) of DNA containing 20 mM NaCl and 1 mM EDTA were added 10  $\mu\text{g/ml}$  of labeled 8-methoxypsoralen or labeled psoralen. Measured volumes (4 ml) of the prepared solutions were introduced into calibrated glass tubes immersed in a thermostatically controlled bath and irradiated for different periods by means of a HPW 125 Philips lamp (which emits almost exclusively at 365 nm) at a distance of 7.5 cm. The total radiation intensity incident on the 4 ml of the solution, determined by using a chemical actinometer [26] corresponded to  $9.6 \times 10^{15}$  quanta/sec. After irradiation each solution was divided into 2 portions: 2 ml were chromatographed on Sephadex, solution A, while the remaining 2 ml were utilized for hydroxylapatite column chromatography, solution B.

#### Gel Filtration

2 ml of the solution A were introduced into a column (20  $\times$  1.5 cm) of Sephadex G-25 and then eluted with 20 mM NaCl, 2 mM EDTA aqueous solution, collecting fractions of 3.5 ml with a flow rate of 15 drops/min. In each fraction the absorbance at 260 nm and the radioactivity were determined.

#### Determination of Cross-Linkages

The determination of cross-linkages was performed either on the basis of the renaturation capacity shown by DNA after heat denaturation [27,28] or on the basis of the increase of the single stranded DNA molecular weight.

In the first case 2 ml of each solution B were heated for 10 min in a boiling water bath and then immersed in ice for 15 min; this solution was then chromatographed on a column (0.7  $\times$  4 cm) of hydroxylapatite Bio-Gel type (Bio-Rad Laboratories, Calif., U.S.A.) and developed using a linear gradient of 0.05–0.3 M phosphate buffer (pH 6.98). Fractions of 3.5 ml were collected using an LKB Ultrarac fraction collector, with a flow rate of 15 drops per min. In each fraction the absorbance at 260 nm was determined; on the basis of the amounts of single stranded (noncross-linked) and of renatured double stranded (cross-linked) DNA separated and determined in this way, the extent of cross-linkage formation was evaluated.

In the second case from the samples of the polynucleotides solutions, irradiated in the presence of 8-MOP, 100  $\mu\text{l}$  were withdrawn and mixed with an equal volume of a 0.5 N NaOH solution containing 1% sucrose [in the case of poly d(C-G).poly d(C-G) 2% sucrose]; the samples were then gently layered on to linear 5–20% sucrose gradient [10–40% for poly d(C-G).poly d(C-G)], prepared in 0.1 N NaOH, which, after holding at 5° C for 30 min, were centrifuged at 38,000 rpm, in a SW 39 rotor at the same temperature, using a Spinco Model L centrifuge. Gradients were then fractionated, the sucrose concentration and the absorbance at 260 nm were determined.

The number of cross-linkages produced per polynucleotide molecule (N) was calculated by the following equation [29]:

$$N = \frac{Mn^*}{Mn} - 1$$

where Mn is the average number molecular weight [30] of the untreated polynucleotide and Mn\* is the average number molecular weight of the polynucleotide irradiated in the presence of 8-MOP. Molecular weights were calculated according to Studier [31].

## RESULTS

### Fluorescent properties of 8-MOP

Although psoralen derivatives generally have strong fluorescent properties, 8-MOP has a relatively low fluorescence when excited with long wavelength ultraviolet light. In Fig 2 the excitation and emission spectra are reported; the exciting  $\lambda_{\text{max}}$  is 348 nm; the fluorescent  $\lambda_{\text{max}}$  is 501 nm. In comparison with psoralen (the parent unsubstituted furocoumarin which has exciting  $\lambda_{\text{max}} = 338$  nm and fluorescent  $\lambda_{\text{max}} = 445$  nm), an aqueous solution  $1.4 \times 10^{-4}$  M of 8-MOP in 0.2 M phosphate buffer pH = 6.98 has a fluorescence intensity 20 times lower than that of an equivalent solution of psoralen.

### Binding Parameters of the Molecular Complex with DNA

It has been well ascertained that psoralens in their ground state form molecular complexes with DNA involving very weak bonds [23,32], in which the psoralen molecules are intercalated between 2 base pairs [23]. The binding process between various psoralen derivatives, including 8-MOP, and DNA has already been studied [23]; the data obtained have now been re-elaborated according to the recently published method of Mc Ghee and Von Hippel [25]; the thus-calculated binding isotherm of 8-MOP is reported in Fig 3, together with that of psoralen for comparison. From this treatment the value of K [intrinsic association constant to an isolated site, according to Mc Ghee and Von Hippel [25]] was  $7.36 \times 10^2$  and the value of  $1/n$  (frequency of the binding sites; in other words the number of molecules bound per nucleotide of DNA) was 0.128. The corresponding values of psoralen were  $3.97 \times 10^2$  and 0.108, respectively.

These data indicate a relatively low affinity of 8-MOP for DNA in the formation of the molecular complex. For instance, it was determined that in a 0.1% solution of DNA, 8-MOP, added in a concentration of 10  $\mu\text{g/ml}$ , only 58% is complexed with DNA.

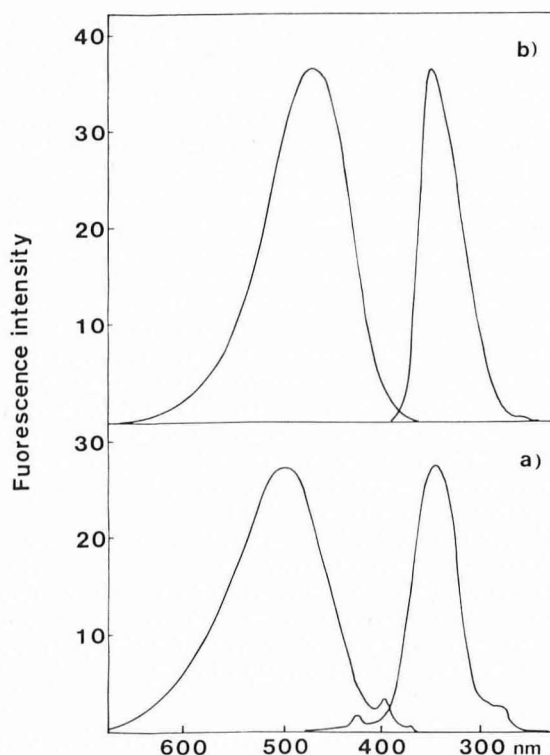


FIG 2. Excitation and fluorescence spectra of 8-MOP ( $1.4 \times 10^{-4}$  M in phosphate buffer pH 6.98) (part a) and of 4',5'-dihydro-8-MOP ( $0.46 \times 10^{-6}$  M in phosphate buffer pH 6.98) (part b).

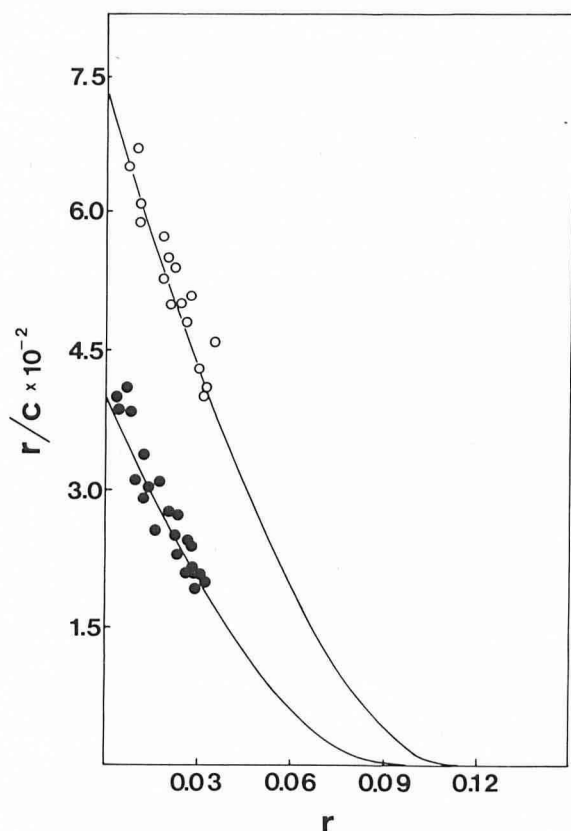


FIG 3. Binding of 8-MOP (—○—) and of psoralen (—●—) in the ground state to DNA; the data are presented in the form of Scatchard plot where  $r$  is the binding ratio (furocoumarin molecules bound per nucleotide) and  $c$  is the free drug concentration. The curves have been computed according to the method of Mc Ghee and Von Hippel.

#### Photobinding with DNA

By irradiating with UVA a solution of DNA plus 8-MOP, the fraction of the drug molecules which are intercalated into DNA photoreact with the pyrimidine bases giving a covalent stable conjugation. To study the photobinding of 8-MOP to DNA, the tritium labeled compound was used; after irradiation DNA was separated from the excess of unbound 8-MOP by means of gelfiltration and the radioactivity covalently linked to the macromolecule was determined. The results are reported in Fig 4; in the same Figure, for comparison, the results obtained in analogous experiments with psoralen are also reported. The photobinding capacity of 8-MOP was found to be only a little lower than that of psoralen.

The results thus obtained had reference to the amounts of drug linked to DNA, without distinction of the mode of binding. In previous studies concerning the photoreaction between psoralen and DNA, the formation of various types of photocycloadducts has been clearly demonstrated. Monoadducts (1 psoralen molecule plus 1 pyrimidine base) of 2 types may take place, according to the psoralen site involved in the cycloaddition: 3,4-monoadducts, which are practically nonfluorescent when observed under Wood's light, and 4',5'-monoadducts which, by contrast, are strongly fluorescent in the same conditions [1]. Furthermore, diadducts (1 psoralen molecule plus 2 pyrimidine bases) may be formed; in this case an interstrand cross-linkage takes place in DNA. In Wood's light diadducts are not fluorescent [2].

Therefore, in the case of psoralen, as well as various other furocoumarin derivatives, such as 4,5',8-trimethyl-psoralen (TMP), after irradiation DNA shows a violet fluorescence, and by measuring its fluorescence intensity it has been possible to

determine the amount of 4',5'-monoadducts formed by irradiation [33].

In the case of 8-MOP, only a very weak fluorescence can be detected in DNA after irradiation. This fact may be due either to the presence of very small amounts of 4',5'-monoadducts or, more likely, to very weak fluorescent properties of these monoadducts. To investigate these possibilities, we have studied the fluorescent properties of 4',5'-dihydro-8-methoxy-psoralen, which would have practically the same properties as the 4',5'-monoadducts (an analogous fact was ascertained in 4',5'-dihydro-psoralen, which has both spectrophotometric and fluorescent spectra practically identical with those of the 4',5'-monoadducts psoralen-thymine [33]); excitation ( $\lambda_{\text{max}} = 352 \text{ nm}$ ) and fluorescent ( $\lambda_{\text{max}} = 472 \text{ nm}$ ) spectra are shown in Fig 2; on equimolecular basis and in the same condition, the fluorescence intensity of 4',5'-dihydro-8-methoxy-psoralen proved to be 80 times lower than that of 4',5'-dihydro-psoralen.

#### Cross-linkage Formation

The formation of interstrand crosslinkages by photoreaction of 1 molecule of 8-MOP with 2 pyrimidine bases appertaining to the opposite strands of DNA, has been evaluated on the basis of the renaturing capacity of the heat-denatured cross-linked DNA, according to a method described by Lawley and Brookes [27]; the separation of the renatured fraction from the irreversibly denatured one was performed by means of column chromatography on hydroxylapatite. The results obtained are reported in Fig 5, together with the results of analogous determinations worked out using psoralen, as a comparison compound.

By subtracting the amounts of 8-MOP forming cross-linkages in DNA from the total amounts of 8-MOP linked to the same DNA, it has been possible to calculate the amounts of 8-MOP present in DNA in the form of monofunctional adducts. The results are reported in Fig 5. It is evident that from a quantitative point of view monoadducts represent the main product of the photoreaction both in the case of 8-MOP and in that of psoralen, while bifunctional adducts represent only a secondary product; the ratio between crosslinkages and monoadducts is about 1:9.

#### Photobinding and Crosslinkage Formation in Various Synthetic Duplex Polydeoxyribonucleotides

To obtain indications of the possible presence, in DNA, of specific receptor sites for the photobinding of 8-MOP, some synthetic double stranded polydeoxyribonucleotides having

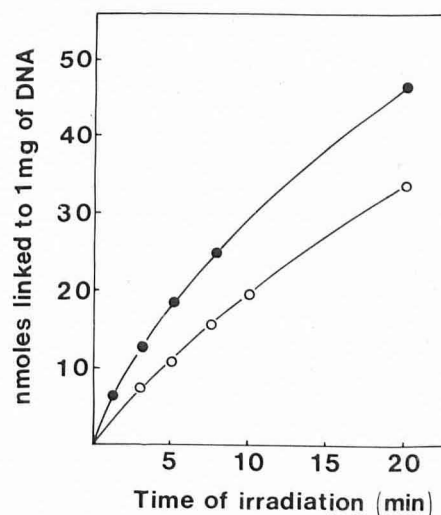


FIG 4. Photobinding of 8-MOP (—○—) and of psoralen (—●—) to calf thymus DNA.



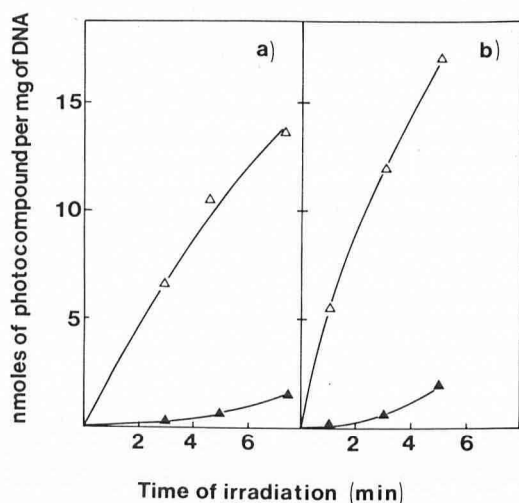


FIG 5. Monofunctional ( $\Delta$ -) and bifunctional ( $\blacktriangle$ -) adducts formed in the photobinding of 8-MOP (part a) and of psoralen (part b) to DNA.

known regularly repeating sequences are chosen and their photoreacting capacity in respect to 8-MOP was studied. Namely, 3 polymers having in each strand an alternate sequence of purine and pyrimidine bases have been used: poly d(A-T).poly d(A-T), poly d(C-G).poly d(C-G) and poly d(A-C).poly d(G-T). Moreover, 2 polymers having in one strand a continuous sequence of pyrimidines and in the other strand a continuous sequence of purine bases have also been used: poly d(A).poly d(T) and poly d(C).poly d(G).

Two different evaluations have been made after the photo-reaction with 8-MOP: (a) the extent of the total photobinding, determined by means of experiments analogous to those described when calf thymus DNA was used; (b) the amount of crosslinkages. In the latter case, only the 3 polydeoxyribonucleotides having alternate sequences of purines and pyrimidines in each strand have been used; in fact, those having linear sequences cannot form crosslinkages. The extent of crosslinkages has been determined by sedimentation experiments in alkaline sucrose gradients, evaluating the mean increase of the molecular weight; actually, in this case hydroxylapatite chromatography of heat-denatured polymers cannot be used, because after cooling these polydeoxyribonucleotides undergo a complete spontaneous renaturation.

In this case, too, by subtracting the amount of 8-MOP forming crosslinkages from the total amount of 8-MOP linked to the same polynucleotide, it has been possible to calculate the amount of 8-MOP forming monofunctional adducts.

The results of the total photobinding experiments are reported in Fig 6. 8-MOP showed the highest photobinding capacity towards poly d(A-T).poly d(A-T) and a gradually decreasing one towards poly d(A-C).poly d(G-T) and poly d(C-G).poly d(C-G) respectively. The two polymers having in each strand a continuous sequence of purines and pyrimidines (poly dA.poly dT and poly dC.poly dG) showed the lowest photo-reactivity.

In Fig 7 the amounts of crosslinkages formed by 8-MOP in the various polynucleotides having an alternate sequence of purines and pyrimidines in each strand are reported as a function of the A-T percentage, together with the amounts of monofunctional adducts formed in the same polynucleotides. We can observe the different shapes of the curves; in fact, while the highest number of monofunctional adducts occurred in poly d(A-T).poly d(A-T) (A-T = 100%), the highest number of crosslinkages took place in poly d(A-C).poly d(G-T) having an A-T content of 50%; in those having only A-T (100%) or only C-G (A-T = 0%) the extent of crosslinkages was dramatically decreased.

### Photobinding and Crosslinkage Formation in Various DNA Samples Having Different Base Pair Composition

In order to confirm the data obtained with synthetic polydeoxyribonucleotides, the photobinding capacity of 8-MOP and its ability to form crosslinkages have been studied in respect to various DNA samples having different base pair composition. Four DNA samples have been examined, other than that from calf thymus (A-T content 58.1%) used in previously reported studies: DNA from *Clostridium perfringens* (A-T = 69%), from

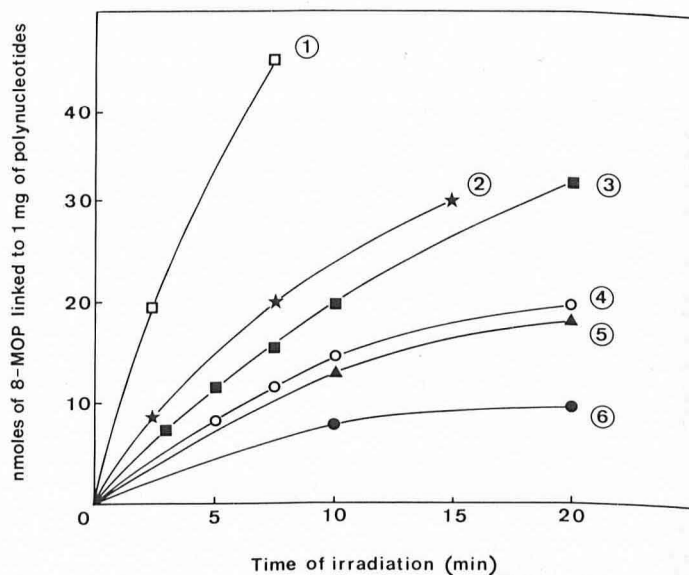


FIG 6. Photobinding of 8-MOP to various polydeoxyribonucleotides: poly d(A-T).poly d(A-T) : 1; poly d(A-C).poly d(G-T) : 2; poly d(C-G).poly d(C-G) : 4; poly d(A).poly d(T) : 5; poly d(C).poly d(G) : 6; calf thymus DNA (for comparison):3.

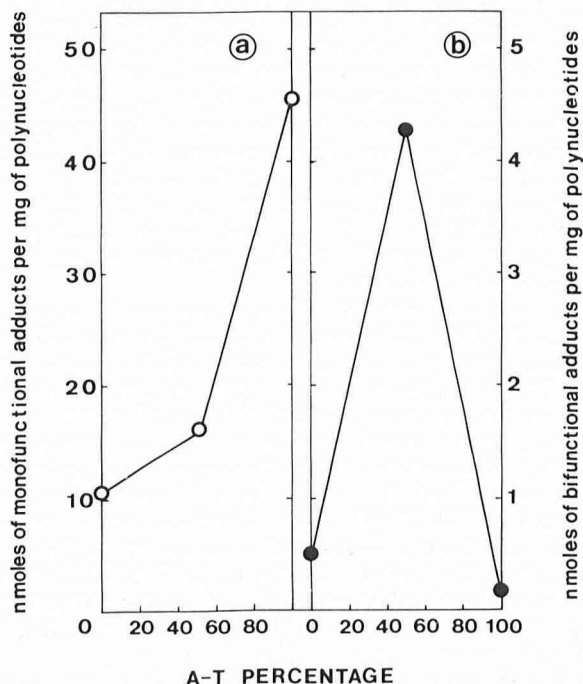


FIG 7. Monofunctional (part a) and bifunctional (part b) adducts formed in the photobinding of 8-MOP to polydeoxyribonucleotides having in each strand alternate sequences of purines and pyrimidines, as a function of A-T percentage, after 7.5 minutes of irradiation.

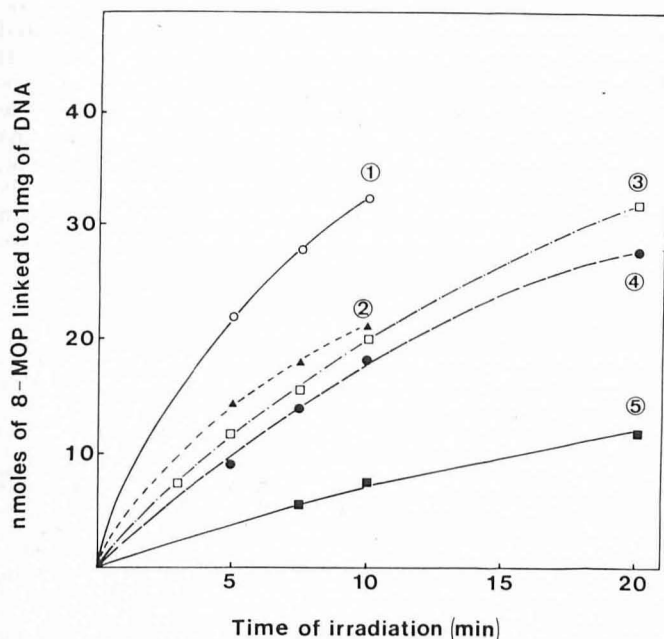


FIG 8. Photobinding of 8-MOP to various DNA samples having different base composition: DNA from *Clostridium perfringens* (1); DNA from salmon sperm (2); DNA from calf thymus (3); DNA from *E. coli* (4); DNA from *Micrococcus lysodeikticus* (5).

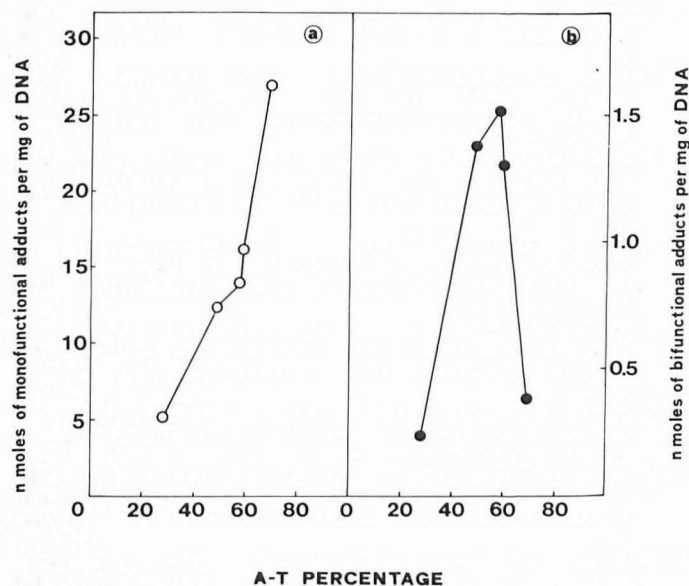


FIG 9. Monofunctional (part a) and bifunctional (part b) adducts formed in the photobinding of 8-MOP with various DNA samples having different base composition, reported as a function of A-T percentage, after 7.5 min of irradiation.

salmon sperm (A-T = 58.8%), from *E. coli* (A-T = 49.9%) and from *Micrococcus lysodeikticus* (A-T = 28.1%).

The data of the total photobinding experiments are reported in Fig 8. In Fig 9 the amounts of crosslinkages formed in the various DNA samples are reported as a function of the A-T percentage contained in the same DNA samples, together with the amounts of monofunctional adducts formed in the various samples, calculated as previously mentioned. A good parallelism exists between the extent of monoaddition of 8-MOP and the A-T percentage contained in the DNA samples, showing a behaviour analogous to that observed using synthetic polydeoxyribonucleotides. By contrast, the highest number of crosslinkages (evaluated by hydroxylapatite chromatography) oc-

curred in DNA samples containing about equal amounts of A-T and C-G (DNA from calf thymus, salmon sperm and *E. coli*); when the content of A-T was much lower or higher, the formation of crosslinkages decreased noticeably.

## DISCUSSION

Some fundamental aspects of the interaction between 8-MOP and DNA have been investigated; the results obtained are described in this paper. In some cases, the behavior of 8-MOP is compared with that of psoralen (see molecular structure in Fig 1); in fact, this compound is the parent, nonsubstituted linear furocoumarin and although it has never been clinically used for the therapeutic treatment of skin diseases, it has been extensively investigated in basic studies concerning the photobiological properties of furocoumarins and the interaction with DNA. Concerning the correlation between the chemical structure and the photobiological properties it has often been taken as a reference compound.

It is well known that 2 types of interaction may occur between a furocoumarin and DNA: (a) formation of a molecular complex, in the dark, consisting of an intercalation of a furocoumarin molecule, in its ground state, between 2 base pairs of DNA, involving only very weak bonds; (b) photochemical reaction, following irradiation with UVA light, leading to a covalent combination of the furocoumarin molecule to pyrimidine bases of DNA. Both these aspects have been investigated.

The studies of the complex formation between 8-MOP in its ground state (without any irradiation) and DNA showed a low affinity of 8-MOP for the macromolecule, although a little higher than that of psoralen. This means that a notable fraction of the molecules present in the solution remains free and the molecular complex is easily dissociable.

This property may have significance in the photochemotherapy of psoriasis and other skin diseases (PUVA therapy); in fact, when 8-MOP is administered orally, it is distributed throughout the whole body, but only at the skin level can it be reached by UVA radiation, which provokes the covalent photobinding to DNA and the consequent photosensitization. In all internal parts of the body, where UVA radiation cannot arrive, no photoreaction with DNA can take place, but eventually only the formation of a molecular complex. The weakness and easy dissociability of this complex, now observed *in vitro*, minimize the possible risk deriving from this type of interaction. We point out in fact, that many drugs are known to have antibacterial, antitumor and mutagenic activity as a consequence of the formation of molecular complexes with DNA (actinomycin, daunomycin, adriamycin, etc), however in these cases very strong complexes are formed *in vitro* with DNA, having much higher values of the association constant (3 to 5 orders of magnitude higher) and of the number of molecules which can be linked to the macromolecule [34-38].

While generally psoralens have strong fluorescent properties when excited with UVA radiation, 8-MOP appeared to emit only a weak fluorescence, 20 times lower than that of psoralen. Furthermore DNA, while after the photobinding of some psoralen molecules it acquires a strong violet fluorescence, due to the formation of 4',5'-cycloadducts [33,39,40], after the photobinding of 8-MOP molecules acquired only a very weak fluorescence. In fact, 4',5'-dihydro-8-methoxy-psoralen, which can be assumed as a model compound of the 4',5'-cycloadduct, having the same chromophoric moiety, showed a very weak fluorescent emission, 80 times lower than that of 4',5'-dihydropsoalene, compared to an equimolecular basis.

The very weak fluorescence acquired by DNA makes it difficult to follow the photobinding of 8-MOP to DNA on the basis of fluorescence measurements, and explain what has been reported by Meffert et al [41], who by examining with fluorescence microscopy slices of guinea pig skin previously treated with 8-MOP and irradiated with UVA, observed that fluorescence was lacking in the nuclei, while present in the cytoplasm,

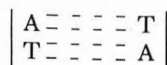
concluding that 8-MOP under irradiation binds to proteins and not to nucleic acids. The very low fluorescent properties of 4',5'-cycloadducts of 8-MOP indicate that the lack of fluorescence in the nuclear DNA cannot be correlated to the lack of photobinding of 8-MOP.

In covalent photobinding to DNA, 8-MOP behaved like many other linearly condensed furocoumarins (psoralens), that is forming mainly monoadducts with the pyrimidine bases of DNA and also crosslinkages; the ratio between the mono- and difunctional adducts was found to be 9:1 in calf thymus DNA. It is known that mono- and difunctional adducts (crosslinkages) may be differently able to produce biological effects [39,42]. In fact, while inhibition of DNA synthesis in living cells appeared to be correlated with the total photobinding of the furocoumarin derivatives (monoadducts plus crosslinkages), in other cases, such as killing of bacteria [4], inactivation of tumor cells [43], erythema formation on skin [1-3], it was demonstrated that the intensity of the effects can well be correlated only with the formation of crosslinkages [4,10,39,42-44].

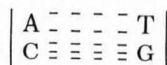
To obtain indications whether the covalent photobinding of 8-MOP occurs at random along the macromolecule or, alternatively, specific receptor sites are present, photobinding and crosslinking formation have been studied using some synthetic double stranded polydeoxyribonucleotides having known regularly repeating sequences, chosen as model compounds, as well as some DNA samples extracted from different sources, having different contents of A-T and C-G pairs.

In the experiments with polynucleotides, monofunctional adducts (calculated by subtracting crosslinkages from the total photobinding) were formed in the highest amount with poly d(A-T).poly d(A-T), and in a gradually decreasing one with the other polynucleotides (see Fig 7). The results obtained with the DNA samples having different base composition were in agreement with these; in fact, as Fig 9 shows, monofunctional photobinding of 8-MOP was parallel with the A-T content of the same DNA samples.

These results seem to indicate that monofunctional addition of 8-MOP takes place preferentially at the level of DNA regions having a structure corresponding to that of poly d(A-T).poly d(A-T); therefore, the preferential site of DNA for the monofunctional addition of 8-MOP seems to be the following sequence:



We have also studied the ability of 8-MOP to form crosslinkages both with the various synthetic polynucleotides and with the DNA samples having different base pair composition. However, the results obtained in this case were not parallel with those previously described, obtained studying the monofunctional addition. In fact (see Fig 9), the highest number of crosslinkages was formed by 8-MOP when it photoreacted with poly d(A-C).poly d(G-T), while with poly d(A-T).poly d(A-T) and with poly d(C-G).poly d(C-G) it was notably lower. In the various DNA samples, the highest amount of crosslinkages occurred when A-T and C-G were present to about the same extent; in the samples containing both a higher and a lower content of A-T the formation of crosslinkages was remarkably lower. These results are in agreement with the suggestion that for the formation of crosslinkages by 8-MOP the following sequence is preferred:



In conclusion, the results obtained suggest that two different preferential sites are present in DNA for the photobinding of 8-MOP, one for the formation of monofunctional adducts and another for the formation of crosslinkages. This suggestion may appear not surprising, when we consider that an influence of the structure of the bases present in the sequence of DNA on

the position assumed by the 8-MOP molecule intercalated between them seems possible. On the other hand, from various experimental evidence, the position assumed by the furocoumarin molecule in respect to the pyrimidine bases appears to play an important role in determining the possibility and the modality of its photoreaction [40,45]. An analogous behavior, leading to analogous conclusions has been observed recently also in the photoreactions of psoralen and 8-methylpsoralen with various DNA samples having different base composition [40].

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